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Hair cortisol is not associated with reactogenicity after MMR-vaccination in 6-month-old infants

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Author statement

Dr. Malon conceptualized the study, collected data, performed the data analysis and interpretation of results, and drafted the initial manuscript.

Dr. Jensen performed the data analysis and interpretation of result.

Dr. Vittrup, Dr. Zimakoff, Dr. Sørensen, and Dr. Lind collected the data and revised the manuscript.

Dr. Jørgensen supervised the cortisol analyses and revised the manuscript.

Dr. Svensson conceptualized and designed the study, supervised data collection, and critically revised the manuscript for important intellectual content. Dr. Stensballe conceptualized the study and designed the study, supervised data collection, critically revised the manuscript for important intellectual content and is the guarantor.

The corresponding author attests that all listed authors meet authorship criteria. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

1. Introduction

Most vaccines are administered in childhood and despite being effective in preventing diseases or reducing disease severity, are associated with a risk of adverse reactions (Hervé et al., 2019; Di Pasquale et al., 2016). However, knowledge on the pathogenic mechanisms that are involved in the development of adverse reactions and the potential role of cortisol is incomplete.

Reactogenicity represents the physical manifestation of the inflammatory response to vaccination and includes injection site pain, redness, swelling, and induration, as well as systemic symptoms (Hervé et al., 2019; Di Pasquale et al., 2016). Reactogenicity is primarily driven by the innate immune system and mediated by interleukin (IL)-1, IL-6, tumor necrosis factor-alpha (TNF-α), and prostaglandin E2. All of these have pyrogenic qualities, with the latter being the central mediator of raised body temperature and systemic symptoms such as headache, myalgia, and chills (Hervé et al., 2019; Saper et al., 2012; Konsman et al., 2002). That being said, not all individuals experience reactogenicity and the risk of adverse reactions is generally quite low. Indeed work by Hong et al. indicated that local adverse reactions, such as pain or itching at the injection site, in the seven days after vaccination against measles were only observed in 3.8% of infants aged 6–8 months. Similarly, systemic adverse reactions, such as fever, were observed in 6.7% of the same cohort (Hong et al., 2019).

Factors with possible impact on reactogenicity include host factors such as age and sex, vaccine factors such as injection route (e.g., subcutaneous or intramuscular), vaccine type and adjuvants, and administration factors including administration route (e.g., oral or injection), needle length and injection speed (Hervé et al., 2019). One example of a host factor effect is that the youngest children tend to exhibit fewer injection site reactions than adults, but are more susceptible to exhibit systemic reactions such as febrile episodes. This may be explained by higher levels of pro-inflammatory cytokines (e.g., IL-6 and IL-10) in children after vaccination (Hervé et al., 2019). Another is that females experience more reactogenicity than males (Hervé et al., 2019).

A general association between systemic inflammatory mediators and systemic symptoms after immunization is supported in the literature (Hervé et al., 2019). Stress may influence the immune system and the inflammatory response and has been hypothesized to influence reactogenicity through elevated levels of circulating cytokines e.g., IL-6

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Abbreviations:

IL	interleukin
TNF-α	tumor necrosis factor alpha HCC = hair cortisol concentration
MMR	measles, mumps, and rubella
SAE	serious adverse events
ELISA	enzyme-linked immunosorbent assay
GA	gestational age
HR	hazard ratio
RR	risk ratio
RD	risk difference

(Hervé et al., 2019; Dhabhar, 2014; Glaser and Kiecolt-Glaser, 2005). In response to psychological or biological stressors, the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system are activated (McEwen, 2007). Regulated by cortisol and catecholamines, the stress response aims to adapt the body and mind to stressful situations. Overall, cortisol is known to inhibit inflammation and to induce a shift from the production of pro-inflammatory to anti-inflammatory cytokines (T_H1 to T_H2 response) (Steptoe et al., 2007). However, the intensity and duration of cortisol exposure may impact the immune response. Acute stress (lasting minutes to hours) has been suggested to provide an immune-enhancing effect, which is associated with the redistribution of leukocytes to the blood and skin, mucosa of the gastrointestinal and urogenital tracts, lung, liver, and lymph nodes in preparation for potential stressor-mediated immune challenges (Dhabhar, 2014). In contrast, persistent stress (lasting weeks to years) has been associated with suppression or dysregulation of immune function (Dhabhar, 2009, 2014; Dragoş and Tănăsescu, 2010), which may lead to an increased susceptibility to infections and diseases such as cancer and depression (Dhabhar, 2014; Glaser and Kiecolt-Glaser, 2005). Interestingly, an increased level of circulating IL-6 has been associated with both acute and persistent stress (Glaser and Kiecolt-Glaser, 2005; Steptoe et al., 2007; Marsland et al., 2017).

In our literature search, we only detected two studies investigating the suggested association between reactogenicity and stress, neither of which included infants or measured a biological marker of persistent stress. In the first study, perceived stress was not associated with injection site reactogenicity after vaccination against human papillomavirus (107 females aged 14–45 years and five males, aged 14–26 years) (Petousis-Harris et al., 2013). In the second study (59 male students aged 18–30 years), a significant increase in IL-6 was reported in the subgroup randomized to vaccination (typhoid vaccine) and stress (2 × 5 min performing a challenging mental task 30 min after vaccination). No significant change in the rating of somatic symptoms or body temperature was found (Brydon et al., 2009).

Measurement of hair cortisol concentration (HCC) is increasingly used to estimate persistent stress in neonates, children, and adults (Bates et al., 2017; Karlen et al., 2015; Palmer et al., 2013; Liu and Doan, 2019; de Kruijff et al., 2020; Yamada et al., 2007; Russell et al., 2012). Cortisol is thought to diffuse into the hair shaft passively and can be reliably measured in the proximal 3–6 cm of hair (1 cm represents onemonth) (Bates et al., 2017; Russell et al., 2012; Gray et al., 2018).

In this study, we aimed to investigate if there is an association between HCC and reactogenicity reported by parents after experimental measles, mumps, and rubella (MMR) vaccination or placebo at 5–7 months of age.

2. Methods and material

Hair samples were collected in mother-child pairs by cutting hair close to the scalp from those who had provided biological samples during their participation in the double-blinded Danish MMR trial (Vittrup et al., 2020). Injection with randomly assigned MMR vaccine (M-M-RVaxPro) or placebo (vaccine solvent) was administered in the infant's anterolateral region of the thigh. Randomization was generated in the online system REDCap in a 2:4:6 block stratified by site, sex, and prematurity and blinded until the last randomization and completed data validation. Information was collected regarding frequency of hair wash (categorized as 0–1, 2, and 3+ times per week), use of hair dye within the past two months, or use of hair styling products (including baby oil) within 24 h prior to collection of the sample.

2.1. Registration of adverse reactions and adverse events

At inclusion, participants were informed about possible adverse reactions following immunization by specially trained study staff.

This information was repeated on the day of the intervention and participants received a paper diary (Supplementary, S1) for notes. Based on known reactogenicity, reported in other trials and listed in the M-M-RVaxPro insert package (Di Pietrantonj et al., 2020; Summary of product characteristics, 2015), the diary included: a list of predefined symptoms, *adverse reactions* (expected to happen in more than 1/1000 of participants), and two rare conditions with greater severity (febrile seizures and thrombocytopenia) (Vittrup et al., 2020) (Fig. 1). The parents were asked to tick off the boxes and to register time to first onset if they experienced any of these predefined symptoms.

Beyond the adverse reactions, any additional symptom observed by the parents as *adverse events* was registered in the diary as free text. Parents were asked to describe the symptomatology, onset, and course (Fig. 1). Severity of all reactogenicity data collected was assessed with severe adverse events (SAE) defined as adverse events resulting in hospitalization >24 h, prolonged hospitalization, life-threatening condition, or death.

Parent reports were collected by telephone approximately six weeks post-intervention except in cases of SAE's, where parents were encouraged to contact the study staff as soon as possible (Vittrup et al., 2020).

2.2. Exposure and outcome

The primary exposure was infant HCC, and the secondary exposure was maternal HCC. As no prior knowledge of infant HCC in our specific age group was available, we chose to analyze the primary and secondary exposures both as continuous (log_2-scale) and binary variables (high vs. low HCC). For the binary variable, we defined high- and low-HCC as above and below the median value, respectively. The analyses were performed separately by randomization group (MMR and placebo).

The primary outcome was all registered adverse reactions, measured as time from intervention to symptom onset and combined into one composite outcome. Co-primary outcomes were the composite measure adjusted for covariates and effect modification.

The secondary outcomes were adverse events, measured as time from intervention to symptom onset and categorized by organ system (Fig. 1), and SAE's, which were categorized separately.

Children without events were censored on the day of the phone call or 49 days after the intervention (whichever came first).

2.3. Stress analyses

We used a competitive enzyme-linked immunosorbent assay (ELISA) to measure levels of HCC. The most proximal three cm of the hair samples collected was scissored into small pieces. For the extraction process, 1 ml of methanol was added, the mixture was sonicated for 30 min and then incubated for 20 h at a 52 °C shaker (300 rpm). The following day, the methanol was evaporated to dryness with nitrogen at 45 °C and stored at −20 °C until analysis. Before analysis, the samples were thawed to room temperature, then again left to evaporate until they reached a dryness of 45 °C with nitrogen (for 10 min). Thereafter

Very common: ≥1/10
Injection site erythema, pain, and/or swelling
Fever (38.5˚C or higher)
Common: ≥1/100 to <1/10
Rash and urticaria
Injection site bruising
Uncommon: ≥1/1000 to ≤1/100
Nasopharyngitis / upper respiratory tract infection
Rhinorrhoea
Diarrhoea or vomiting

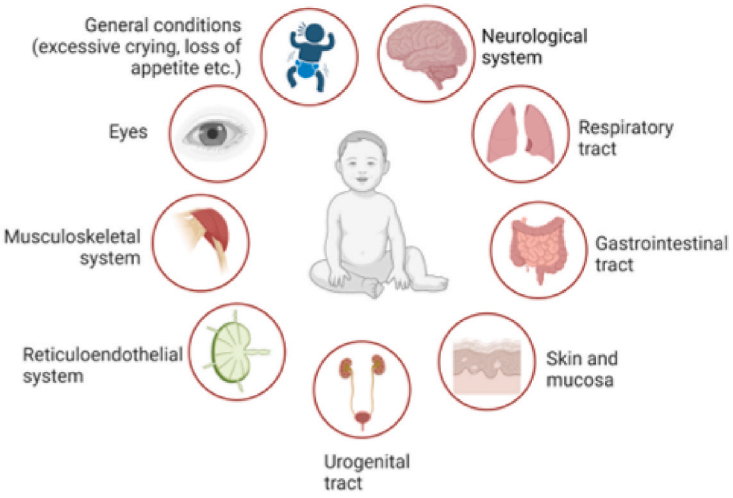

Fig. 1. Left: Adverse reactions after M-M-RVaxPro defined as known reactogenicity. Expected frequencies are indicated. Right: Adverse events defined as all reported symptoms besides adverse reactions, categorized by organ system.

they were dissolved in 500 µL phosphate-buffered saline buffer pH 8.0, mixed, and centrifuged at 2000 rpm for 2 min.

Samples were analyzed using the Cortisol (Saliva) ELISA assay (Alpco.com, Salem, NH, USA) according to the manufacturer's instructions. If results were outside the range of the standard curve (>40 ng/ml), samples were diluted and analyzed as such (to avoid extrapolation of the standard curve). The lower level of detection was specified to be 0.15 ng/ml. The intermediary precision was determined using internal controls (patient samples) as 16.7% (at 0.22 ng/ml), 11.4% (at 9.8 ng/ml), and 7.7% (at 49.2 ng/ml). All samples were analyzed in duplicate, and results were converted from ng/ml to pg cortisol/mg hair (corrected concentration × mL PBS buffer/mg hair × 1000).

2.4. Covariates and effect modifiers

Covariates were: sex (male/female), prematurity (defined as gestational age (GA) < 37 weeks), infant age at vaccination (days), maternal age at vaccination (years), number of siblings, maternal education, household income and maternal level of HCC. The two age variables were analyzed as continuous variables. We categorized the number of siblings (0, 1, 2, or 3+), maternal education ("short higher-education" or below, bachelor's degree, master's degree, or research degree), and household income (< USD 56,775/year and ≥ USD 56,775/year). The covariates were included in the adjusted analyses as one joint set of variables. In addition, sex was included as an effect modifier. The covariates and the effect modifier were chosen based on the findings from other studies (Gray et al., 2018; Karlén et al., 2013; Bryson et al., 2021; Perry et al., 2022). Only analyses for the primary outcome (composite outcome of adverse reactions) were analyzed using covariates and effect modification to reduce the number of analyses.

2.5. Statistical analysis

Stata SE 17.0 and R version 4.1.0 were used for statistical analyses. The analyses were based on the per-protocol principle, i.e., infants who did not follow the allocation were excluded.

Cortisol concentration values were $\log_2$-transformed in the continuous exposure analyses. However, the crude HCC values for the groups are presented in Table 2.

Hazard ratios (HR) for the continuous and binary exposure were estimated by Cox regression. Based on the latter model, risk ratios (RR) and risk differences (RD) for the binary exposure (high vs. low HCC) were obtained (Ozenne et al., 2020). For the continuous exposure analyses, linearity was examined by comparing the cumulative martingale residuals to 1000 simulated processes under the model (Ozenne et al., 2020). Outliers were inspected graphically but included in the analyses. However, analyses were repeated for the primary outcomes and two secondary outcomes with the highest number of events as *post-hoc* analyses, using two different methods for handling outliers: 1) exclusion of values < the 25th percentile - 1.5* Inter Quartile Range (IQR) and values > the 75th percentile + 1,5*IQR and 2) exclusion of values < mean – 3SD and values > mean + 3SD.

Values below 0.15 mg/ml were imputed using the uniform distribution over the interval from 0 to 0.15.

Linear regression was used to examine the association between mother and infant HCC, between HCC and hair wash, hair dye, and hair styling products, and between infant HCC and sex, prematurity, age at vaccination, maternal age, household income, maternal education, and the number of siblings (Martinussen and Scheike, 2006).

A statistical analysis plan of the present study was conducted and stored with the trial Data Safety Monitoring Board before the laboratory analyses were initiated. The significance levels were adjusted for multiplicity. Any supplemental analyses were denoted *post-hoc* analyses (Malon, Jensen).

3. Ethics

The trial was approved by the Danish Medicines Agency (EudraCT 2016-001901-18. J.no. 2016103428), the Committees on Biomedical Research Ethics (H-16041195), and the Capital Region Data Regulation Unit (RH-2017-247, I-Suite no.: 05714). The trial was registered at ClinicalTrials.gov (NCT03780179) (CDC. ClinicalTrials, 2021). Written informed consent was collected from caregivers. No significant differences in the risk of reactogenicity have been reported between MMR vaccination in infants below nine months and at nine months or older (Nic Lochlainn et al., 2019).

4. Results

4.1. Baseline characteristics

In total, hair samples from 692 mother-infant pairs were collected (n = 57 infants and n = 1 mother excluded due to insufficient hair growth on the infant or parents refused collection of hair samples). Additionally, 36 infant samples were excluded: one child received the wrong intervention; in 33 cases analyses were missing or failed (e.g., broken tubes, insufficient sealing, or material); and in two cases information regarding adverse reactions was missing (n = 2). In mothers, 16 analyses were

excluded because of missing or failed analyses (n = 2 of these also failed in the corresponding infant sample). Therefore, 562 infant-mother pairs were eligible for analysis (n = 243 in the MMR group, n = 319 in the placebo group) (Fig. 2). Baseline characteristics are presented in Table 1. The frequency of reported adverse events is published separately.

4.2. Hair cortisol characteristics

HCC in infants ranged from 4.3 to 188,201 pg/mg (Table 2). HCC in mothers ranged from 1.5 to 9543 pg/mg (Table 2).

Box plots for log_2-transformed HCC for infants and mothers are presented in Fig. 3.

No association was found between the level of HCC and the number of hair washes in either infants (β = −0.05 [−0.21; 0.05]) or mothers (β = −0.01 [−0.13; 0.10]).

The use of hair dye or styling products was not analyzed in infants due to few observations (n = 0 and n = 15, respectively). HCC in mothers was not associated with the use of hair dye or styling products (β = 0.03 [−0.36; 0.75] and β = −0.03 [−0.54; 0.23], respectively).

HCC in infants and mothers was associated (β = 0.15 [0.01; 0.31], p < 0.001) (Fig. 4).

In contrast, no association was found for infant HCC and sex (β = 0.04 [−0.17; 0.51]), prematurity (β = 0.08, [−0.09; 1.40]), infant age at vaccination (β = −0.07 [−0.03; 0.00]), maternal age at vaccination (β = −0.08 [−0.08;-0.00]), siblings (0 siblings β = −0.04 [−0.54; 0.22]; 1 sibling β = −0.04 [−0.74; 0.29]; 2 siblings β = −0.07 [−2.03; 0.17]; 3+ siblings β = −0.02 [−1.71; 1.16]), maternal education (bachelor's degree β = 0.02 [−0.41; 0.60], master's degree or researcher degree β = −0.04 [−0.63; 0.25]) or household income (≥USD56,775, β = 0.0006 [−0.45; 0.45]).

Table 1
Baseline characteristics.

	MMR n = 243	Placebo n = 319
	N and (%) for categorical variables Mean and [range] for continuous variables	N and (%) for categorical variables Mean and [range] for continuous variables
Gender		
Male	130 (53)	161 (50)
Female	113 (46)	158 (50)
Prematurity GA<37 + 0	19 (7)	12 (3)
Missing	2 (1)	10 (3)
Age at vaccination		
Child mean (months)	6.4 [5.2–7.0]	6.4 [5.1–7.0]
Mother mean (years)	33.3 [23–47]	33.1 [23–46]
No. Of sibling		
0	115 (48)	162 (51)
1	81 (34)	102 (32)
2	34 (14)	42 (13)
3+	10 (4)	12 (4)
Missing	3 (1)	1 (0)
Educational status mother		
Short higher education or below	51 (21)	62 (19)
Bachelor's degree	62 (26)	77 (24)
Master's degree or Research degree	127 (52)	178 (56)
Missing	3 (1)	2 (1)
Household income pr. year		
<56,775 USD	37 (15)	58 (18)
≥56,775 USD	201 (83)	257 (80)
Missing	5 (2)	4 (2)

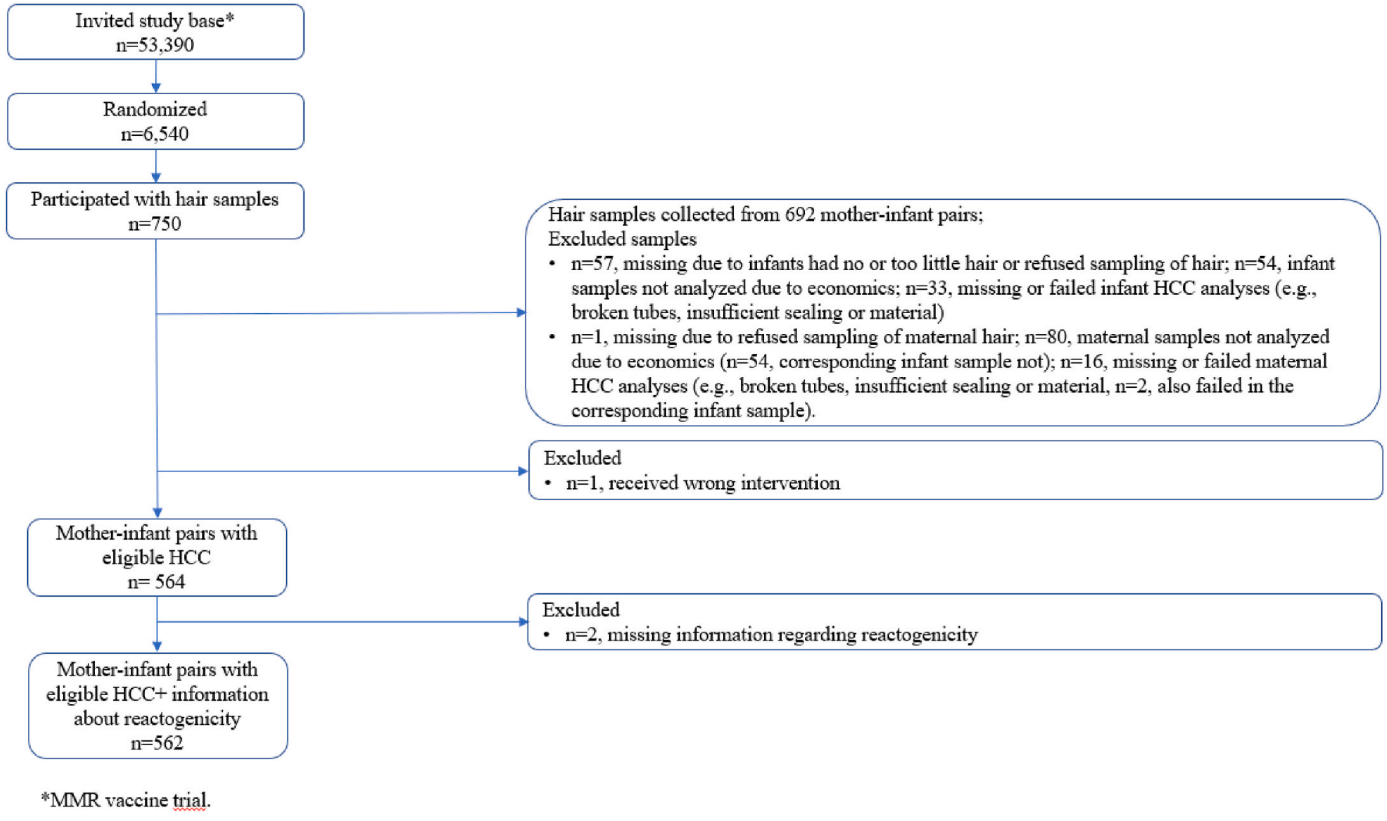

Fig. 2. Flow diagram of the progress of participants through the study.

Table 2
HCC characteristics; non-logaritmized data.

	HCC pg/mg	Obs	Mean	Median	Range
Infant	MMR	243	2701.9	549.0	[4.3; 115,666.7]
	Placebo	319	2698.6	563.3	[15.6; 188,201.4]
	All	562	2700.0	561.4	[4.3; 188,201.4]
Mother	MMR	243	210.1	99.0	[1.8; 9542.9]
	Placebo	319	179.2	91.5	[1.5; 4126.1]
	All	562	192.6	97.0	[1.5; 9542.9]

4.3. Associations between the level of HCC and reactogenicity

We found that neither infant nor maternal HCC was associated with the parent-reported reactogenicity in any of the analyses (Table 3). Moreover, neither effect modification of sex nor adjustment for covariates nor *post-hoc* analyses with the exclusion of outliers (Supplementary, S2) changed the results.

Fig. 5 shows the cumulative risk for developing the composite event over time in infants with high HCC vs. low HCC.

In this cohort of 562 mother-child pairs, no cases of febrile seizures,

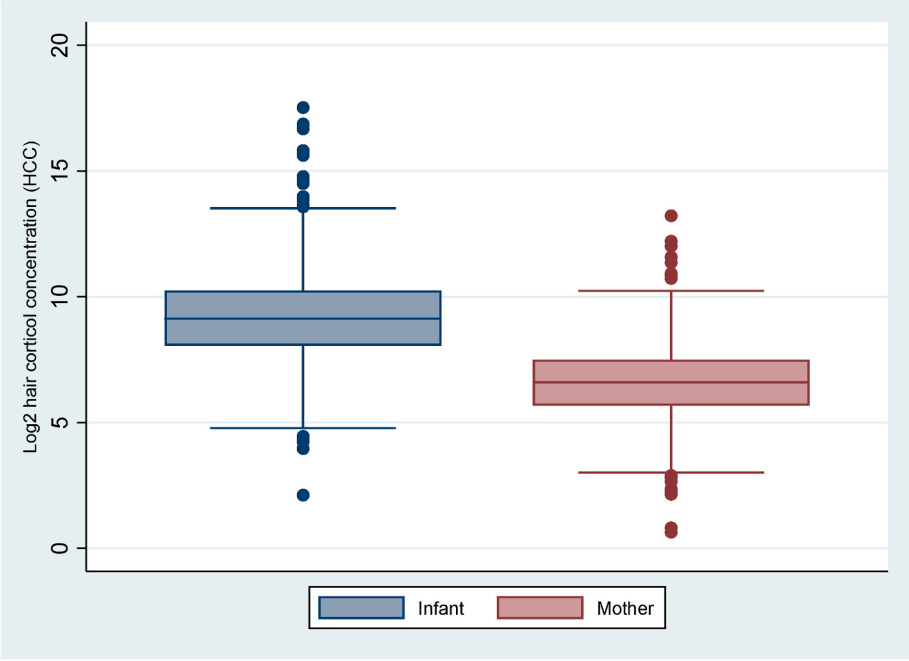

Fig. 3. Box plots of hair cortisol concentration for all infants (left) and all mothers (right) at the intervention date. Boxes indicate Inter Quartile Range (IQR); points indicate outliers.

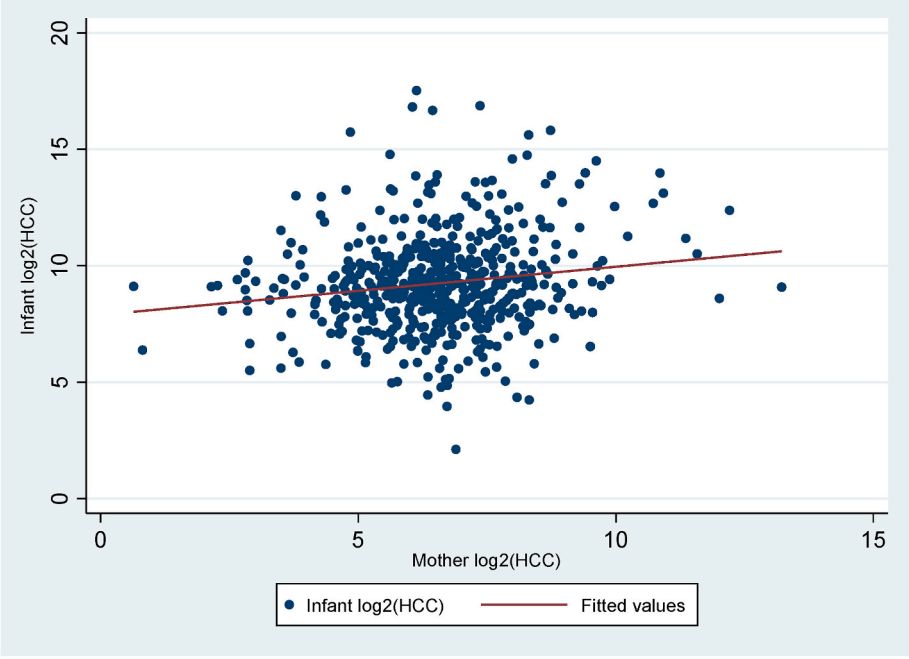

Fig. 4. Scatter plot of the log-transformed hair cortisol concentration in infants and mothers.

Table 3
Vaccine group; HR and 95% CI are reported for Cox regression analyses, RR, RD, and 95% CI for the g-formula method.

	Events/ n	HR [95% CI]	p	HR [95% CI]	p	RR [95% CI]	p	RD [95% CI]	p
Primary analysis, infant HCC exposure		log_2(HCC)		low vs. high HCC		low vs. high HCC		low vs. high HCC	
Composite measure[a]	151/ 243	1.00 [0.92; 1.09]	0.92	1.05 [0.77; 1.45]	0.75	1.03 [0.85; 1.26]	0.80	0.02 [-0.10; 0.14]	0.80
- Effect modification by sex[b]	151/ 243	1.01 [0.88; 1.17]	0.86	0.98 [0.62; 1.58]	0.96	–	–	–	–
Composite measure – adjusted[c]	151/ 243	1.00 [0.91; 1.10]	0.99	1.03 [0.74; 1.46]	0.85	–	–	–	–
Secondary analyses, infant HCC exposure									
Adverse events relating to general conditions (excessively crying, eating less etc.)	13/243	0.84 [0.63; 1.11]	0.22	0.65 [0.21; 2.00]	0.46	–	–	–	–
Adverse events relating to the airways	2/243	1.27 [0.73; 2,21]	0.40	–	–	–	–	–	–
Adverse events relating to ears, nose, and/or throat	12/243	0.90 [0.67; 1.20]	0.46	0.74 [0.23; 2.32]	0.60	–	–	–	–
Adverse events relating to the gastrointestinal tract	4/243	1.21 [0.80; 1.83]	0.37	1.07 [0.15; 7.59]	0.95	–	–	–	–
Adverse events relating to the skin and mucosa	38/243	1.01 [0.87; 1.18]	0.87	1.41 [0.62; 2.21]	0.63	1.22 [0.81; 2.32]	0.26	0.05 [-0.03; 0.13]	0.26
Adverse events relating to symptoms from the eyes	1/143	0.86 [0.31; 2.37]	0.77	–	–	–	–	–	–
Adverse events relating to symptoms from the urogenital system	1/243	0.57 [0.25; 1.27]	0.17	–	–	–	–	–	–
Adverse events relating to symptoms from the reticuloendothelial system (RES)	1/243	0.82 [0.30; 2.25]	0.70	–	–	–	–	–	–
Adverse events relating to symptoms from the musculoskeletal system	0	–	–	–	–				
Serious adverse events (SAE)	1/243	0.81 [0.30; 2.20]	0.68	–	–	–	–	–	–
Secondary analyses, maternal HCC exposure									
Composite measure[a]	151/ 243	1.03 [0.94; 1.14]	0.53	1.20 [0.87; 1.66]	0.26	1.12 [0.92; 1.33]	0.24	0.06 [-0.05; 0.17]	0.25

[a] Composite outcome; see text for details.
[b] Effect modifier = sex (male/female), female as the reference group.
[c] 'Set of covariates'; see text for details.

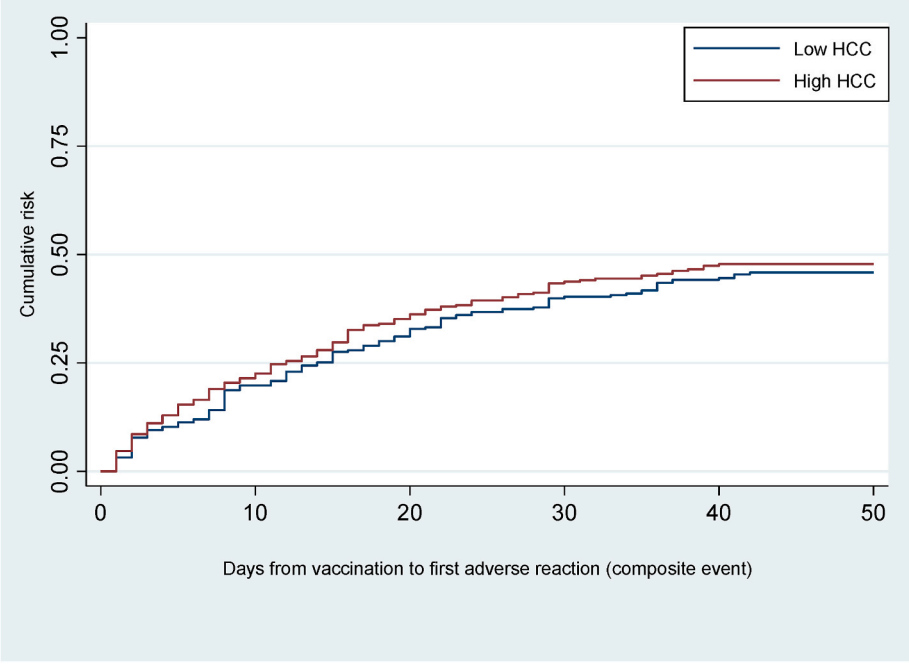

Fig. 5. The cumulative risk for high- and low levels of HCC, the composite outcome for infants in the MMR group.

thrombocytopenia, or neurological symptoms were reported. Only a few cases were reported regarding general symptoms and symptoms from the respiratory tract, the eyes, the urogenital system, the reticuloendothelial system, the musculoskeletal system, and serious adverse events. Therefore, it was not feasible to fit the models for these outcomes.

No evidence against the assumption of a linear relationship between the continuous HCC exposure and the log-hazard of the outcomes was found (e.g., composite measure unadjusted $p = 0.73$, Fig. 6) using the martingale residuals.

HR and RR in the MMR group were similar (Table 3) to the placebo group (Table 4). The exclusion of 23 maternal hair samples, incubated for 40 h instead of the planned 20 h, did not change the results.

5. Discussion

Our results do not suggest any association between maternal or infant stress measured as HCC and parent-reported reactogenicity in infants after experimental MMR vaccination or placebo. This conclusion is in accordance with the two previous studies (Petousis-Harris et al., 2013; Brydon et al., 2009), although our larger study uses a different stress measurement and covers a different age group.

Adverse reactions following immunization are related to the inflammatory response. The biology of the local and systemic immunological response is well described, albeit the role of cortisol is less clear (Hervé et al., 2019). However, our findings indicated a limited role of cortisol in the immune system in terms of reported reactogenicity in this specific age group. In the present study, we did not conduct analyses of cytokines. Reactogenicity from MMR is most frequently observed 7–10 days after immunization (Sundhedsstyrelsen and Danish He alth Authority, 2023). As reactogenicity was similar in the MMR and placebo groups, the association between HCC and true reactogenicity of the MMR vaccine may be difficult to detect. Thus, we question whether we are observing reactogenicity, a mix, or just everyday events occurring in infants unrelated to MMR. Nevertheless, infant HCC do not seem to influence these types of events.

Moreover, we did not find that the level of maternal HCC impacted reported reactogenicity, excluding a significant effect of overreporting or underreporting by stressed mothers. We have not identified any other studies on the association between maternal HCC and reactogenicity. We speculated that more stressed mothers worry about adverse events, which may lead to overreporting, and/or could be less observant and overlook symptoms, which may lead to underreporting. However, our results do not support these hypotheses as we found no under- or over-reporting in those with high HCC levels.

In our study population, we found a small but significant association between infant and maternal HCC. This has also been reported in other studies (Karlén et al., 2013; Perry et al., 2022). In accordance with other studies, the infantile HCC measurement in our data showed a wide range, suggesting a greater biological variation of HCC at early age (Bates et al., 2017; Liu and Doan, 2019; de Kruijff et al., 2020; Karlén et al., 2013; Liu et al., 2016), perhaps due to immature regulation (Liu and Doan, 2019). Indeed, in a study in 1-year-old children, HCC levels ranged from 0.18 to 1667 pg/mg hair (Karlén et al., 2013). However, Yamada et al. (2007) demonstrated that HCC was significantly higher in hospitalized term neonates than healthy neonates.

Additionally, in a subgroup of term neonates admitted to the NICU needing ventilation, the authors found that HCC increased with the number of days in ventilation. This suggests that HCC also reflects persistent infant stress despite the biological variation. Other factors such as oxytocin might counteract or modify the effects of a high cortisol level (Miller et al., 2011; Milaniak et al., 2017), but this has not been measured in any of the previous studies concerning reactogenicity. Furthermore, genetic factors may be important (Tucker-Drob et al., 2017), yet few studies have linked genes to reactogenicity (Feenstra et al., 2014; Klein et al., 2021). We found that the distributions of maternal and infant HCC were shaped similarly, although the range of the former was narrower. Our results contrast reports from other studies on mothers and expectant mothers, where lower values and narrower ranges have been described (Karlén et al., 2013; D'Anna-Hernandez et al., 2011; Tarullo et al., 2017). Variations in the methodological handling of outliers may provide explanation for inter-study differences - for example, excluding outliers more than three standard deviations from the mean prior to analysis (Perry et al., 2022). Still, our post-hoc analyses, excluding outliers by two different methods, did not change the results.

HCC has been shown to increase during pregnancy and decrease after birth (D'Anna-Hernandez et al., 2011). It has been speculated that cortisol levels in mothers might depend on the life adjustments relating to motherhood, such as infant caretaking, sleep disruption, and deprivation (Liu and Doan, 2019).

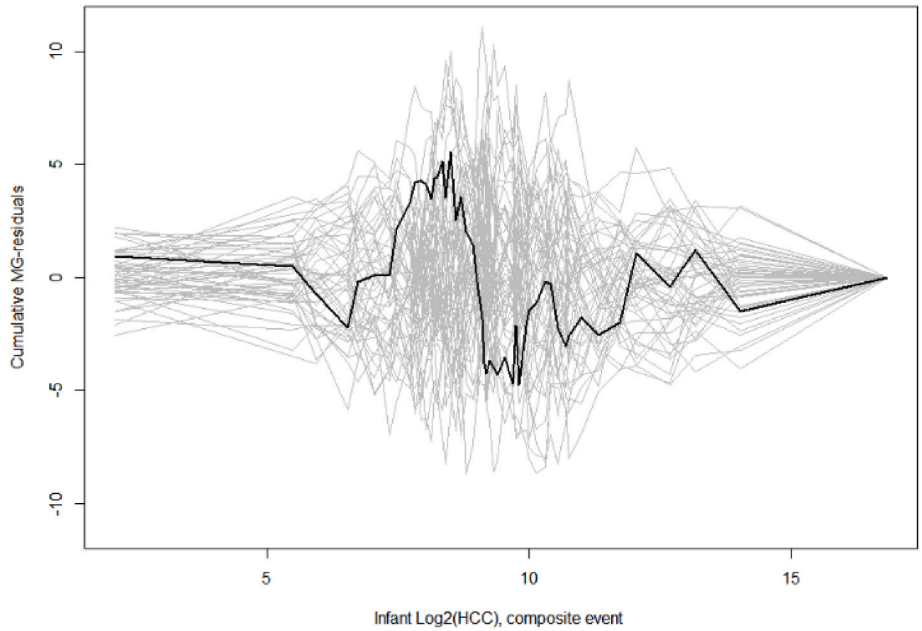

Fig. 6. Linearity plot for the composite outcome, infants in the MMR group.

Table 4
Placebo group; HR and 95% CI are reported for Cox regression analyses, RR, RD, and 95% CI for the g-formula method.

	Events/ n	HR [95% CI]	p	HR [95% CI]	p	RR [95% CI]	p	RD [95% CI]	p
Primary analysis, infant HCC exposure		log_2(HCC)		low vs. high HCC		low vs. high HCC		low vs. high HCC	
Composite measure[a]	193/ 319	0.95 [0.89; 1.02]	0.15	0.78 [0.58; 1.03]	0.08	0.86 [0.71; 1.01]	0.07	−0.09 [-0.19; 0.01]	0.07
- Effect modification by sex[b]	193/ 319	0.91 [0.82; 1.00]	0.06	0.74 [0.49; 1.21]	0.16	–	–	–	–
Composite measure – adjusted[c]	193/ 319	0.95 [0.88; 1.02]	0.15	0.76 [0.56; 1.03]	0.07	–	–	–	–
Secondary analyses, infant HCC exposure									
Adverse events relating to general conditions (excessively crying, eating less etc.)	15/319	1.10 [0.88; 1.38]	0.41	0.86 [0.31; 2.37]	0.77	–	–	–	–
Adverse events relating to symptoms from the airways	4/319	1.09 [0.70; 1.70]	0.71	–	–	–	–		–
Adverse events relating to ears, nose, and/or throat	20/319	1.10 [0.91; 1.34]	0.33	2.36 [0.91; 6.14]	0.08	–	–	–	–
Adverse events relating to symptoms from the gastrointestinal tract	7/319	0.96 [0.66; 1.39]	0.83	0.74 [0.16; 3.29]	0.69	–	–	–	–
Adverse events relating to symptoms from the skin and mucosa	50/319	1.14 [1.00; 1.30]	0.04	1.69 [0.79; 2.43]	0.25	1.39 [0.88; 2.15]	0.16	0.07 [-0.03; 0.17]	0.16
Adverse events relating to symptoms from the eyes	2/319	1.34 [0.79; 2.27]	0.28	–	–	–	–	–	–
Adverse events relating to symptoms from the urogenital system	1/319	1.12 [0.47; 2.68]	0.80	–	–	–	–	–	–
Adverse events relating to symptoms from the reticuloendothelial system (RES)	0/319	–	–	–	–	–	–	–	–
Adverse events relating to symptoms from the musculoskeletal system	1/319	0.57 [0.19; 1.69]	0.31	–	–				
Serious adverse events (SAE)	0/319	–	–	–	–	–	–	–	–
Secondary analyses, maternal HCC exposure									
Composite measure[a]	193/ 319	0.93 [0.85; 1.02]	0.11	0.76 [0.57; 1.01]	0.06	0.85 [0.70; 1.01]	0.06	−0.10 [-0.20; 0.00]	0.06

[a] Composite outcome; see text for details.
[b] Effect modifier = sex (male/female), female as the reference group.
[c] Set of covariates'; see text for details.

Consistent with the literature, we found that HCC was not associated with sex, frequency of hair wash, or use of hair dye/styling products in mothers (Gray et al., 2018). Nor was it linked to parental education and income (Liu and Doan, 2019; Bryson et al., 2021). However, the relatively high-income profiles in our population may reduce the power to detect an influence of socioeconomic status.

Other stressors in infancy could be childhood vaccinations, for example, vaccination against diphtheria, tetanus, pertussis, polio, Haemophilus influenzae, and pneumococci, which in Denmark is scheduled at 3, 5, and 12 months of age (Statens Serum Institut (SSI), 2022). The vaccine coverage equals 96% or higher and has been stable during the study period (Sundhedsstyrelsen, 2022). Hence, most infants participating in our study were vaccinated at 3 and 5 months of age. If the prior vaccinations are experienced as stressors in some infants, this would only contribute to more variation in HCC. Worth noting is that the study was partly conducted during the COVID-19 pandemic. Thus, pandemic-related factors might have increased psychological stress in the families. Opposingly, it is also possible that families might have felt less stressed because of, for example, less demand for social activities. Thus, the COVID-19 pandemic and prior vaccination might have resulted in a higher number of individuals with high or low values of HCC, but this would increase our scope to investigate the influence of hair cortisol on reactogenicity.

5.1. Strengths and limitations

The strengths of the study are the large sample size and the placebo-controlled randomized clinical trial design. Moreover, reports of reactogenicity were detailed and with a high feedback rate (99.5%). However, some caveats should be considered when interpreting our findings. Length of hair was not registered before analysis; hence the duration of the stress (if 1 cm of hair represents one month) exposure prior to sample collection cannot be interpreted (Bates et al., 2017). However, we have not found studies showing cortisol to influence hair length in this age group, and thus, any misclassification ought to be random. Another limitation of the study is that information regarding the use of corticosteroids was not collected. Use of corticosteroids is rare in our age group, except for topical steroids for atopic dermatitis. However, in a recently published study, corticosteroids was not associated with HCC (Perry et al., 2022) (no distinction between topical, inhalation, and systemic steroids). Lastly, we only studied 5–7 months old infants. The possible effect of hair cortisol on reactogenicity in other age groups is still unknown.

6. Conclusions and perspectives

HCC showed no association with the reported adverse reactions or reported events following experimental MMR vaccination or placebo in infants 5–7 months of age. Infantile HCC showed a wide range which supports a greater biological variation at an early age.

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Declaration of competing interest

o All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

o This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

o The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

Data availability

The investigators on the trial will have access to the final trial dataset. In 2025, access to the deidentified data collection will be available for other researchers.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2023.100626.

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